

On page 4, please replace the second through fourth paragraphs with the following rewritten paragraphs:

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--Figures 1A-1D show the cDNA sequence and corresponding deduced amino acid sequence of the mature NTT polypeptide. The standard one-letter abbreviations are utilized to represent the amino acid residues in the polypeptide sequence shown in Figures 1A-1D.

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptide having the deduced amino acid sequence of Figures 1A-1D, collectively, or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75713 on March 18, 1994. This deposit is a biological deposit with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, USA.

The polynucleotide of this invention was discovered in a cDNA library derived from a human fetal brain. It is structurally related to the neurotransmitter transporter family. It contains an open reading frame encoding a protein of about 727 amino acid residues. The protein exhibits the highest degree of homology to a rat neurotransmitter transporter (NTT4) with 94% identity and 96% similarity over the entire amino acid sequence.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in Figures 1A-1D, collectively, or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same, mature polypeptide as the DNA of Figures 1A-1D, collectively, or the deposited cDNA.--

[Starting on page 4, please replace the paragraph bridging pages 4 and 5 with the following rewritten paragraph:]

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--The polynucleotide which encodes for the mature polypeptide of Figures 1A-1D, collectively, or for the mature polypeptide encoded by the deposited cDNA may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.--

[On page 5, please replace the second through fourth full paragraph with the following rewritten paragraphs:]

--The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figures 1A-1D, collectively, or the polypeptide encoded by the cDNA of the deposited clone. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in Figures 1A-1D, collectively, or the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of Figures 1A-1D, collectively, or the polypeptide encoded by the cDNA of the deposited clone. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figures 1A-1D, collectively, or of the coding sequence of the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.--

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121213 10125 14223 20123 24121 30123 34121 38123 42121 46123 50121 54123 58121 62123 66121 70123 74121 78123 82121 86123 90121 94123 98121 102123 106121 110123 114121 118123 122121 126123 130121 134123 138121 142123 146121 150123 154121 158123 162121 166123 170121 174123 178121 182123 186121 190123 194121 198123 202121 206123 210121 214123 218121 222123 226121 230123 234121 238123 242121 246123 250121 254123 258121 262123 266121 270123 274121 278123 282121 286123 290121 294123 298121 302123 306121 310123 314121 318123 322121 326123 330121 334123 338121 342123 346121 350123 354121 358123 362121 366123 370121 374123 378121 382123 386121 390123 394121 398123 402121 406123 410121 414123 418121 422123 426121 430123 434121 438123 442121 446123 450121 454123 458121 462123 466121 470123 474121 478123 482121 486123 490121 494123 498121 502123 506121 510123 514121 518123 522121 526123 530121 534123 538121 542123 546121 550123 554121 558123 562121 566123 570121 574123 578121 582123 586121 590123 594121 598123 602121 606123 610121 614123 618121 622123 626121 630123 634121 638123 642121 646123 650121 654123 658121 662123 666121 670123 674121 678123 682121 686123 690121 694123 698121 702123 706121 710123 714121 718123 722121 726123 730121 734123 738121 742123 746121 750123 754121 758123 762121 766123 770121 774123 778121 782123 786121 790123 794121 798123 802121 806123 810121 814123 818121 822123 826121 830123 834121 838123 842121 846123 850121 854123 858121 862123 866121 870123 874121 878123 882121 886123 890121 894123 898121 902123 906121 910123 914121 918123 922121 926123 930121 934123 938121 942123 946121 950123 954121 958123 962121 966123 970121 974123 978121 982123 986121 990123 994121 998123 1002121 1006123 1010121 1014123 1018121 1022123 1026121 1030123 1034121 1038123 1042121 1046123 1050121 1054123 1058121 1062123 1066121 1070123 1074121 1078123 1082121 1086123 1090121 1094123 1098121 1102123 1106121 1110123 1114121 1118123 1122121 1126123 1130121 1134123 1138121 1142123 1146121 1150123 1154121 1158123 1162121 1166123 1170121 1174123 1178121 1182123 1186121 1190123 1194121 1198123 1202121 1206123 1210121 1214123 1218121 1222123 1226121 1230123 1234121 1238123 1242121 1246123 1250121 1254123 1258121 1262123 1266121 1270123 1274121 1278123 1282121 1286123 1290121 1294123 1298121 1302123 1306121 1310123 1314121 1318123 1322121 1326123 1330121 1334123 1338121 1342123 1346121 1350123 1354121 1358123 1362121 1366123 1370121 1374123 1378121 1382123 1386121 1390123 1394121 1398123 1402121 1406123 1410121 1414123 1418121 1422123 1426121 1430123 1434121 1438123 1442121 1446123 1450121 1454123 1458121 1462123 1466121 1470123 1474121 1478123 1482121 1486123 1490121 1494123 1498121 1502123 1506121 1510123 1514121 1518123 1522121 1526123 1530121 1534123 1538121 1542123 1546121 1550123 1554121 1558123 1562121 1566123 1570121 1574123 1578121 1582123 1586121 1590123 1594121 1598123 1602121 1606123 1610121 1614123 1618121 1622123 1626121 1630123 1634121 1638123 1642121 1646123 1650121 1654123 1658121 1662123 1666121 1670123 1674121 1678123 1682121 1686123 1690121 1694123 1698121 1702123 1706121 1710123 1714121 1718123 1722121 1726123 1730121 1734123 1738121 1742123 1746121 1750123 1754121 1758123 1762121 1766123 1770121 1774123 1778121 1782123 1786121 1790123 1794121 1798123 1802121 1806123 1810121 1814123 1818121 1822123 1826121 1830123 1834121 1838123 1842121 1846123 1850121 1854123 1858121 1862123 1866121 1870123 1874121 1878123 1882121 1886123 1890121 1894123 1898121 1902123 1906121 1910123 1914121 1918123 1922121 1926123 1930121 1934123 1938121 1942123 1946121 1950123 1954121 1958123 1962121 1966123 1970121 1974123 1978121 1982123 1986121 1990123 1994121 1998123 2002121 2006123 2010121 2014123 2018121 2022123 2026121 2030123 2034121 2038123 2042121 2046123 2050121 2054123 2058121 2062123 2066121 2070123 2074121 2078123 2082121 2086123 2090121 2094123 2098121 2102123 2106121 2110123 2114121 2118123 2122121 2126123 2130121 2134123 2138121 2142123 2146121 2150123 2154121 2158123 2162121 2166123 2170121 2174123 2178121 2182123 2186121 2190

Ab

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figures 1A-1D, collectively, or that encoded by the deposited cDNA, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.--

Starting on page 7, please replace the paragraph bridging pages 7 and 8 with the following rewritten paragraph:

A7
--The fragment, derivative or analog of the polypeptide of Figures 1A-1D, collectively, or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.--

Starting on page 23, please replace the paragraph bridging pages 23-25 with the following rewritten paragraph:

AS --The DNA sequence encoding for NTT, ATCC # 75713 is initially amplified using PCR oligonucleotide primers corresponding to the 5' and sequences of the processed NTT protein (minus the signal peptide sequence) and the vector sequences 3' to the NTT gene. Additional nucleotides corresponding to NTT were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence GACTAAAGCTTGGCATCAATGCCGAAGAAC (SEQ ID NO:3) contains a Hind III restriction enzyme site followed by 18 nucleotides of NTT coding sequence. The 3' sequence GAACTTCTAGAGCAGTGGTCACAGCTCAG (SEQ ID NO:4) contains complementary sequences to Xba I site and is followed by 18 nucleotides of NTT sequence. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 was then digested with Hind III and Xba I. The amplified sequences were ligated into pQE-9 and were inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture was then used to transform E. coli strain M15/rep 4 available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 (O.D.₆₀₀) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by

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Station	Time	Lat.	Long.	Alt.	Temp.	Wind	Clouds	Pressure	Humidity	Visibility	Remarks
1	0000	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
2	0100	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
3	0200	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
4	0300	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
5	0400	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
6	0500	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
7	0600	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
8	0700	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
9	0800	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
10	0900	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
11	1000	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
12	1100	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
13	1200	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
14	1300	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
15	1400	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
16	1500	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
17	1600	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
18	1700	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
19	1800	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
20	1900	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
21	2000	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
22	2100	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
23	2200	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
24	2300	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear
25	0000	33° 32'	113° 10'	2715	55.0	000	000	1013.2	75	10	Clear

Starting on page 25, please replace the paragraph bridging pages 25 and 26 with the following rewritten paragraph:

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The DNA sequence encoding for NTT, ATCC # 75713, was constructed by PCR on the original EST cloned using two primers: the 5' primer GACTAAGATCTGCCACCATGCCGAAGAACAGCAAAGTG (SEQ ID NO:5) contains a Bgl II site followed by 21 nucleotides of NTT coding sequence starting from the initiation codon; the 3' sequence GAACTGATATCGCAGTGGTCACAGCTCAG (SEQ ID NO:6) contains complementary sequences to EcoR V site, translation stop codon, and the last 18 nucleotides of the NTT coding sequence. Therefore, the PCR product contains a Bgl II site, NTT coding sequence followed by a translation termination stop codon, and an EcoR V site. The PCR amplified DNA fragment and the vector, pcDNAI/Amp, were digested with Bgl II and EcoR V. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant NTT, COS cells were transfected with the expression vector by DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the NTT HA protein was detected by radiolabelling and immunoprecipitation method. (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ³⁵S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

All

121.641	121.33	121.02	120.71	120.40	120.09	119.78	119.47	119.16	118.85	118.54	118.23	117.92	117.61	117.30	116.99	116.68	116.37	116.06	115.75	115.44	115.13	114.82	114.51	114.20	113.89	113.58	113.27	112.96	112.65	112.34	112.03	111.72	111.41	111.10	110.79	110.48	110.17	109.86	109.55	109.24	108.93	108.62	108.31	108.00	107.69	107.38	107.07	106.76	106.45	106.14	105.83	105.52	105.21	104.90	104.59	104.28	103.97	103.66	103.35	103.04	102.73	102.42	102.11	101.80	101.49	101.18	100.87	100.56	100.25	99.94	99.63	99.32	99.01	98.70	98.39	98.08	97.77	97.46	97.15	96.84	96.53	96.22	95.91	95.60	95.29	94.98	94.67	94.36	94.05	93.74	93.43	93.12	92.81	92.50	92.19	91.88	91.57	91.26	90.95	90.64	90.33	90.02	89.71	89.40	89.09	88.78	88.47	88.16	87.85	87.54	87.23	86.92	86.61	86.30	85.99	85.68	85.37	85.06	84.75	84.44	84.13	83.82	83.51	83.20	82.89	82.58	82.27	81.96	81.65	81.34	81.03	80.72	80.41	80.10	79.79	79.48	79.17	78.86	78.55	78.24	77.93	77.62	77.31	77.00	76.69	76.38	76.07	75.76	75.45	75.14	74.83	74.52	74.21	73.90	73.59	73.28	72.97	72.66	72.35	72.04	71.73	71.42	71.11	70.80	70.49	70.18	69.87	69.56	69.25	68.94	68.63	68.32	68.01	67.70	67.39	67.08	66.77	66.46	66.15	65.84	65.53	65.22	64.91	64.60	64.29	63.98	63.67	63.36	63.05	62.74	62.43	62.12	61.81	61.50	61.19	60.88	60.57	60.26	59.95	59.64	59.33	59.02	58.71	58.40	58.09	57.78	57.47	57.16	56.85	56.54	56.23	55.92	55.61	55.30	54.99	54.68	54.37	54.06	53.75	53.44	53.13	52.82	52.51	52.20	51.89	51.58	51.27	50.96	50.65	50.34	50.03	49.72	49.41	49.10	48.79	48.48	48.17	47.86	47.55	47.24	46.93	46.62	46.31	46.00	45.69	45.38	45.07	44.76	44.45	44.14	43.83	43.52	43.21	42.90	42.59	42.28	41.97	41.66	41.35	41.04	40.73	40.42	40.11	39.80	39.49	39.18	38.87	38.56	38.25	37.94	37.63	37.32	37.01	36.70	36.39	36.08	35.77	35.46	35.15	34.84	34.53	34.22	33.91	33.60	33.29	32.98	32.67	32.36	32.05	31.74	31.43	31.12	30.81	30.50	30.19	29.88	29.57	29.26	28.95	28.64	28.33	28.02	27.71	27.40	27.09	26.78	26.47	26.16	25.85	25.54	25.23	24.92	24.61	24.30	23.99	23.68	23.37	23.06	22.75	22.44	22.13	21.82	21.51	21.20	20.89	20.58	20.27	19.96	19.65	19.34	19.03	18.72	18.41	18.10	17.79	17.48	17.17	16.86	16.55	16.24	15.93	15.62	15.31	15.00	14.69	14.38	14.07	13.76	13.45	13.14	12.83	12.52	12.21	11.90	11.59	11.28	10.97	10.66	10.35	10.04	9.73	9.42	9.11	8.80	
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In the Drawings:

PF116D1C1